

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

SEQ ID NO 10

**WEST**

## End of Result Set

☐ Generate Collection

13

L2: Entry 1 of 1

File: USPT

Feb 16, 1999

US-PAT-NO: 5872215

DOCUMENT-IDENTIFIER: US 5872215 A

TITLE: Specific binding members, materials and methods

DATE-ISSUED: February 16, 1999

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Osbourne; Jane Katharine	Cambridge			GBX
Allen; Deborah Julie	London			GBX
McCafferty; John Gerald	Babraham			GBX

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE	CODE
Medical Research Council	London			GB2		03
Cambridge Antibody Technology Ltd.	Cambridgeshire			GB2		03

APPL-NO: 8/ 652816

DATE FILED: May 23, 1996

## PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION This application is a continuation-in-part of U.S. patent application Ser. No. 08/244,597, still pending, filed on Jun. 1, 1994, which is the U.S. National Phase of PCT/GB92/02240.

## FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9125582	December 2, 1991
GB	9125579	December 4, 1991
GB	9206318	March 24, 1992
GB	9206372	September 23, 1992
GB	9525004	December 7, 1995
GB	9610824	May 23, 1996

INT-CL: [6] C12P 21/08, C07K 16/32, G01N 33/574

US-CL-ISSUED: 530/387.3; 530/387.5, 530/387.7, 530/388.15, 530/388.85, 530/389.7, 530/391.3, 435/7.23

US-CL-CURRENT: 530/387.3; 435/7.23, 530/387.5, 530/387.7, 530/388.15, 530/388.85, 530/389.7, 530/391.3

FIELD-OF-SEARCH: 530/387.3, 530/387.5, 530/387.7, 530/388.15, 530/388.85, 530/389.7, 530/391.3, 435/7.23

## PRIOR-ART-DISCLOSED:

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO 88/06630	September 1988	WOX	
WO 90/14424	November 1990	WOX	
WO 90/14430	November 1990	WOX	
WO 90/14443	November 1990	WOX	
WO 91/01990	February 1991	WOX	
WO 92/01047	January 1992	WOX	
WO 92/20791	November 1992	WOX	
WO 93/11236	June 1993	WOX	
WO 95/06067	March 1995	WOX	
WO 95/15341	June 1995	WOX	

## OTHER PUBLICATIONS

Bouanani, M. et al., "Autoimmunity to Human Thyroglobulin," Arthritis and Rheumatism, 34(12):1585-1593 (Dec., 1991).

James, K. and Bell, G.T., "Human monoclonal antibody production: Current status and future prospects," Journal of Immunological Methods, 100:5-40 (1987).

Kim, J.G. and Abeyounis, C.J., "Isolation and Characterization of Rat Carcinoembryonic Antigen," Int. Arch. Allergy Appl. Immunol., 92:43-49 (1990).

Kim, J.G. and Abeyounis, C.J., "Monoclonal Rat Antibodies to Rat Carcinoembryonic Antigen," Immunological Investigations, 17(1):41-48 (1988).

Portolano, S. et al., "A Human Fab Fragment Specific for Thyroid Peroxidase Generated by Cloning Thyroid Lymphocyte-Derived Immunoglobulin Genes in a Bacteriophage Lambda Library," Biochemical and Biophysical Research Communications, 179(1):372-377 (Aug. 30, 1991).

Huse et al., "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," Science, 246:1275-1281 (Dec. 8, 1989).

Kang et al., "Linkage of Recombination and Replication Functions by Assembling Combinatorial Antibody Fab Libraries Along Phage Surfaces," Proc. Nat'l Acad. Sci., USA, 88:4363-4366 (May, 1991).

Marks et al., "By-Passing Immunization: Building High Affinity Human Antibodies by Chain Shuffling," Bio/Technology, 10:779-783 (Jul., 1992).

Winter and Milstein, "Man-made Antibodies," Nature, 349:293-299 (Jan. 24, 1991).

Epstein et al., "Production of Carcinoembryonic Antigen From a Human Colon Adenocarcinoma Cell Line II. Use of Monoclonal Antibodies to Carcinoembryonic Antigen For Antigen Purification and Characterization," Developments in Biological Standardization, 66:429-437 (1987).

Griffiths et al., "Human anti-self antibodies with high specificity from phage display libraries," The EMBO J., 12(2):725-734 (1993).

Griffiths et al., "Isolation and high affinity human antibodies directly from large synthetic repertoires," The EMBO J., 13:3245-3260 (1994).

Hu et al., "Minibody: A Novel Engineered Anti-Carcinoembryonic Antigen Antibody Fragment (Single-Chain Fv-H.sub.H 3) Which Exhibits Rapid, High-Level Targeting of Xenografts," Cancer Research, 56(13):3055-3061 (Jul. 1, 1996).

Nap et al., "Specificity and Affinity of Monoclonal Antibodies against Carcinoembryonic Antigen," Cancer Research, 52:2329-2339 (Apr. 15, 1992).

Oikawa et al., "The Carcinoembryonic Antigen (CEA) Contains Multiple Immunoglobulin-Like Domains," Biochemical and Biophysical Research Communication, 144(2):534-542 (Apr. 29, 1987).

Osbourn et al., "Generation of a panel of related human scFv antibodies with high affinities for human CEA," Immunotechnology (Amsterdam) 2(3):181-196 (1996).

Wellerson et al., "Enhanced Binding Activity Observed Between Anti-Carcinoembryonic Monoclonal Antibodies," Hybridoma, 5(3):199-213 (1986).

ART-UNIT: 164

PRIMARY-EXAMINER: Saunders; David  
ASSISTANT-EXAMINER: VanderVegt; F. Pierre  
ATTY-AGENT-FIRM: Marshall, O'Toole, Gerstein, Murray & Borun

**ABSTRACT:**

Specific binding members for human carcinoembryonic antigen (CEA) comprise a human antibody antigen binding domain. The specific binding members may have a dissociation constant less than  $1.0 \times 10^{-8}$  M and may be substantially non-crossreactive with human liver and/or other normal tissues. They may be specific for the A3-B3 extracellular domain of CEA. They may be specific for a carbohydrate epitope of CEA. They may be produced by recombinant expression from encoding nucleic acid and modified and manipulated in various manners in accordance with known techniques. CEA is a tumour antigen and the specific binding members have proven ability to bind and target CEA both in vitro and in vivo.

32 Claims, 42 Drawing figures

SEA ID NO: 10

**WEST**

## End of Result Set

☐ Generate Collection

L1: Entry 1 of 1

File: USPT

Jun 25, 1996

US-PAT-NO: 5530101

DOCUMENT-IDENTIFIER: US 5530101 A

TITLE: Humanized immunoglobulins

DATE-ISSUED: June 25, 1996

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Queen; Cary L.	Los Altos	CA		
Selick; Harold E.	Belmont	CA		

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Protein Design Labs, Inc.	Mountain View	CA			02

APPL-NO: 7/ 634278

DATE FILED: December 19, 1990

## PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS This is a continuation-in-part application of commonly assigned patent application U.S. Ser. No. 07/590,274, filed Sep. 28, 1990 (now abandoned) and of U.S. Ser. No. 07/310,252, filed Feb. 13, 1989 (now abandoned), which is a continuation-in-part of U.S. Ser. No. 07/290,975, filed Dec. 28, 1988 (now abandoned). All of these applications are specifically incorporated herein by reference.

INT-CL: [6] A61K 39/395, C07K 16/28

US-CL-ISSUED: 530/387.3; 530/387.1, 530/388.22, 424/133.1, 424/143.1

US-CL-CURRENT: 530/387.3; 424/133.1, 424/143.1, 530/387.1, 530/388.22

FIELD-OF-SEARCH: 424/85.8, 424/133.1, 424/143.1, 530/387, 530/388.22, 530/387.1, 530/387.3

## PRIOR-ART-DISCLOSED:

## U.S. PATENT DOCUMENTS

Search Selected

Search ALL

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/> 4816397	March 1989	Boss et al.	435/68
<input type="checkbox"/> 4816567	March 1989	Cabilly et al.	530/387
<input type="checkbox"/> 4867973	September 1989	Geers et al.	N/A
<input type="checkbox"/> 5225539	July 1993	Winter	N/A

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0171496	February 1986	EPX	
0173494	March 1986	EPX	
0184187	June 1986	EPX	
0239400	September 1987	EPX	
0266663	June 1988	EPX	
2188941	October 1987	GBX	
WO86/05513	September 1986	WOX	
WO87/02671	May 1987	WOX	
WO89/01783	March 1989	WOX	

## OTHER PUBLICATIONS

Vitteta et al., "Redesigning Nature's Poisons to Create Anti-Tumor Reagents, " Science 238:1098-1104 (1987).

Ellison et al., "The nucleotide sequence of a human immunoglobulin C(gamma).sub.1 gene", Nucleic Acids Res. 10:4071-(1982).

Hieter et al., "Cloned Human and Mouse Kappa Immunoglobulin Constatn and J Region Genes Conserve homology in Functional Segments", Cell 22:197-207 (1980).

Sharon et al., "Expression of a V.sub.H C.sub.K chimaeric protein in mouse myeloma cells", Nature 309:364-367 (1984).

Takeda et al., "Construction of chimaeric processed immunoglobulin genes containing mouse variable and human constant region sequences", Nature 314:452-454 (1985).

Tan et al., "A Human-Mouse Chimeric Immunoglobulin Gene with a Human Variable Region is Expressed in Mouse Myeloma Cells", J. Immunol. 135:3564-3567 (1985).

Morrison et al., "Chimeric human antibody molecules: Mouse antigen-binding domains with human constant region domains," Proc. Natl. Acad. Sci. 81:6851-6859 (1984).

Boulianne et al., "Production of functional chimeric mouse/human antibody, " Nature 312:643-646 (1984).

Neuberger et al., "A hapten-specific chimeric IgE antibody with human physiological effector function," Nature 314:268-270 (1985).

Morrison, S. L., "Transfectomas Provide Novel Chimeric Antibodies," Science 229:1202-1207 (1985).

Sahagan et al., "A Genetically Engineered Murine/Human Chimeric Antibody Retains Specificity for Human Tumor-Associated Antigen", J. Immunol. 137:1066-1074 (1986).

Liu et al., "Expression of mouse::human immunoglobulin heavy-chain cDNA in lymphoid cells", Gene 54:33-40 (1987).

Better et al., "Escherichia coli Secretion of an Active Chimeric Antibody Fragment", Science 240:1041-1043 (1988).

Waldmann, T. A., "The Structure, Function, and Expression of Interleukin-2 Receptors on Normal and Malignant Lymphocytes," Science 232:727-732 (1986).

Leonard et al., "The human receptor for T-cell growth factor," J. Biol. Chem. 260:1872-1880 (1985).

Farrar, J., "The biochemistry, biology, and role of interleukin-2 in the induction of cytotoxic T cell and antibody-forming B cell receptors," Immunol. Rev. 63:129-166 (1982).

Greene et al., "Growth of Human T Lymphocytes: An Analysis of Interleukin 2 and Its Cellular receptor", in Progress in Hematology XIV, E. Brown ed., Grune and Statton, New York (1986) pp. 283-301.

Verhoyen et al., "Reshaping Human Antibodies: Grafting an Antilysozyme Activity", Science 239:1534-1536 (1988).

Jones et al., "Replacing the complementarity-determining regions in a human antibody with those from a mouse", Nature 321:522-525 (1986).

Hale et al., "Remission Induction in Non-Hodgkin Lymphoma with Reshaped Human Monoclonal Antibody CAMPATH-1H", Lancet Dec. 17, 1988, pp. 1394-1399.

Chothia, C. and A. M. Lesk, "Canonical Structures for the Hypervariable Regions of Immunoglobulins", J. Mol. Biol. 196:901-917 (1987).

Reichmann et al., "Reshaping human antibodies for therapy", Nature 332:323-327

(1988).

Bird et al., "Single-chain Antigen-Binding Proteins", Science 242:423-426

(1988).

Huston et al., "Protein engineering of antibody binding sites: Recovery of specific activity in an anti-digoxin single-chain Fv analogue produced in Escherichia coli", Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883 (1988). in Progress in Hematology XIV, E. Brown, ed., Grune and Statton, New York (1986) p. 283.

Kirkman et al. Journal of Expt. Med. vol. 162 Jul. 1985 358.

Uchiyama et al. Journal of Immunology vol. 126 No. 4 1981, 1393.

ART-UNIT: 186

PRIMARY-EXAMINER: Feisee; Lila

ATTY-AGENT-FIRM: Townsend and Townsend and Crew

#### ABSTRACT:

Novel methods for producing, and compositions of, humanized immunoglobulins having one or more complementarity determining regions (CDR's) and possible additional amino acids from a donor immunoglobulin and a framework region from an accepting human immunoglobulin are provided. Each humanized immunoglobulin chain will usually comprise, in addition to the CDR's, amino acids from the donor immunoglobulin framework that are, e.g., capable of interacting with the CDR's to effect binding affinity, such as one or more amino acids which are immediately adjacent to a CDR in the donor immunoglobulin or those within about 3 .ANG. as predicted by molecular modeling. The heavy and light chains may each be designed by using any one or all of various position criteria. When combined into an intact antibody, the humanized immunoglobulins of the present invention will be substantially non-immunogenic in humans and retain substantially the same affinity as the donor immunoglobulin to the antigen, such as a protein or other compound containing an epitope.

13 Claims, 80 Drawing figures

**WEST****End of Result Set**☐ **Generate Collection**

L1: Entry 3 of 3

File: USPT

Jul 30, 1996

US-PAT-NO: 5541110

DOCUMENT-IDENTIFIER: US 5541110 A

TITLE: Cloning and expression of a gene encoding bryodin 1 from Bryonia dioica

DATE-ISSUED: July 30, 1996

**INVENTOR-INFORMATION:**

NAME	CITY	STATE	ZIP CODE	COUNTRY
Siegall; Clay B.	Edmonds	WA		

**ASSIGNEE-INFORMATION:**

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Bristol-Myers Squibb	New York	NY			02

APPL-NO: 8/ 245754

DATE FILED: May 17, 1994

INT-CL: [6] C12N 1/20, C12N 15/63, C12N 9/22, C07H 21/04, C07K 14/00  
US-CL-ISSUED: 435/252.3; 435/320.1, 435/199, 536/23.2, 536/23.6, 530/350  
US-CL-CURRENT: 435/252.3; 435/199, 435/320.1, 530/350, 536/23.2, 536/23.6  
FIELD-OF-SEARCH: 435/69.1, 435/199, 435/320.1, 435/252.3, 536/23.2, 536/23.6, 530/350

**PRIOR-ART-DISCLOSED:****FOREIGN PATENT DOCUMENTS**

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0390040	March 1990	EPX	
2194948	August 1987	GBX	
WO91/00295	January 1991	WOX	

**OTHER PUBLICATIONS**

C. Z. Amorim et al., "Screening of the Antimalarial Activity of Plants of the Cucurbitaceae Family," Mem. Inst. Oswaldo Cruz 86:177-180, 1991.  
Y. Endo et al., "RNA N-Glycosidase Activity of Ricin A-chain," J. Biol. Chem. 262:8128-8130, 1987.  
P.-C. Montecucchi et al., "N-terminal sequence of some ribosome-inactivating proteins," Int. J. Peptide Protein Res. 33:263-267, 1989.  
T. B. Ng et al., "Proteins with abortifacient, ribosome inactivating, immunomodulatory, antitumor and anti-AIDS activities from Cucurbitaceae plants," Gen. Pharmac. 23:575-590, 1992.  
F. Stirpe et al., "Bryodin, a ribosome-inactivating protein from the roots of Bryonia dioica L. (white bryony)," Biochem. J. 240:659-665, 1986.  
F. Stirpe et al., "Modification of ribosomal RNA by ribosome-inactivating



proteins from plants," Nucleic Acids Research 16:1349-1357, 1988.

ART-UNIT: 184

PRIMARY-EXAMINER: Wax; Robert A.

ASSISTANT-EXAMINER: Lau; Kawai

ABSTRACT:

The molecular cloning and expression of biologically active ribosome-inactivating protein bryodin 1 are described. A complete amino acid and oligonucleotide sequence encoding bryodin 1 are also described. Further, plasmids, expression vectors comprising a nucleotide sequence encoding bryodin 1 and transformed host cells are described. Isolation and characterization of the nucleotide sequence for bryodin 1 enables the recombinant production of large amount of bryodin 1 for use in vitro or in vivo directly or as ligand/toxin conjugates or fusion proteins. These compositions can be used to selectively kill undesired cells such as cancer cells, infected cells, bacteria.

11 Claims, 13 Drawing figures

**WEST**☐ Generate Collection

L3: Entry 10 of 15

File: USPT

Aug 26, 1997

US-PAT-NO: 5661016

DOCUMENT-IDENTIFIER: US 5661016 A

TITLE: Transgenic non-human animals capable of producing heterologous antibodies of various isotypes

DATE-ISSUED: August 26, 1997

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lonberg; Nils	San Francisco	CA		
Kay; Robert M.	San Francisco	CA		

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
GenPharm International Inc.	Palo Alto	CA			02

APPL-NO: 8/ 053131

DATE FILED: April 26, 1993

## PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION This application is a continuation-in-part of U.S. Ser. No. 07/990,860 filed Dec. 16, 1992 now U.S. Pat. No. 5,545,806 which is a continuation-in-part of U.S. Ser. No. 07/904,068 filed 23 Jun. 1992, which is a continuation-in-part of U.S. Ser. No. 07/853,408 filed 18 Mar. 1992, which is a continuation in part of U.S. Ser. No. 07/834,539, filed Feb. 5, 1992, which is a continuation-in-part of U.S. Ser. No. 07/810,279 filed Dec. 17, 1991, now U.S. Pat. No. 5,569,825 which is a continuation-in-part of U.S. Ser. No. 07/575,962 filed Aug. 31, 1990, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07/574,748 filed Aug. 29, 1990, now abandoned. This application claims foreign priority benefits under Title 35, United States Code, Section 119, to PCT Application No. PCT/US91/06185 which corresponds to U.S. Ser. No. 07/834,539 filed Feb. 5, 1992 and PCT Application No. PCT/US92/10983.

## FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
WO	PCT/US92/06185	August 28, 1991
WO	PCT/US92/10983	December 17, 1992

INT-CL: [6] C12N 15/00, A61K 39/00, C12P 19/34, C07K 16/00

US-CL-ISSUED: 435/172.3; 424/184.1, 435/91.1, 435/172.1, 530/387.1, 536/23.1, 536/23.53, 800/2, 935/19, 935/89, 935/93, 935/103

US-CL-CURRENT: 435/452; 424/184.1, 435/91.1, 530/387.1, 536/23.1, 536/23.53

FIELD-OF-SEARCH: 800/2, 435/172.2, 435/240.2, 530/388.1

## PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected

Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/>	<u>4643234</u>	February 1987	Moore	530/388
<input type="checkbox"/>	<u>4683195</u>	July 1987	Millis	435/6
<input type="checkbox"/>	<u>4965188</u>	October 1990	Millis	435/6
<input type="checkbox"/>	<u>5047507</u>	September 1991	Buchegger	530/387
<input type="checkbox"/>	<u>5175384</u>	December 1992	Krimpenfort et al.	800/2
<input type="checkbox"/>	<u>5416260</u>	May 1995	Koller	800/2
<input type="checkbox"/>	<u>5434340</u>	July 1995	Krimpenfort et al.	800/2

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0 315 062	May 1989	EPX	
WO 90/04036	April 1990	WOX	
901443	November 1990	WOX	
WO 90/12878	November 1990	WOX	
WO 91/00906	January 1991	WOX	
WO 91/10741	July 1991	WOX	
WO 92/03918	March 1992	WOX	

## OTHER PUBLICATIONS

James et al Journal of Immunological Methods 100:5-40, 1987.  
 Breggenam et al PNAS 86: 6709, 1989.  
 Miller et al Nature 295: 428, 1982.  
 Berman et al EMBO J 7: 727, 1988.  
 Sideras et al International Amweg 1(6); 631, 1989.  
 Rothman et al International Immunology 2(7):621, 1990.  
 Nihoido et al nature 292: 843, 1981.  
 Nihaudo et al. JBC 257: 7322, 1982.  
 Yamamura et al PNAS 83 2152, 1986.  
 Ward et al Nature 341: 544, 1989.  
 Orlandi et al PNAS 86: 3833, 1989.  
 Larrick et al BBNC 160(3): 1250, 1989.  
 Larrick et al In Virto Immuniakia . . . 1988, pp. 231-246.  
 Marks et al J. Mol. Biol. 222: 581, 1991.  
 Clackson et al Nature 352: 624, 1991.  
 Sasty et al PNAS 86: 5728, 1989.  
 Winter et al Nature 349: 293, 1991.  
 Marx Science 246: 1250, 1989.  
 Alt et al., Immunoglobulin genes in transgenic mice, TIG--Aug. 1985.  
 Berman et. al., Content and organization of the human Ig V.sub.H locus: definition of three new V.sub.H families and linkage to the Ig C.sub.H locus, The EMBO J. 7:727-738 (1988).  
 Berton et. al., Synthesis of germ-line .gamma.1 immunoglobulin heavy-chain transcripts in resting B cells: Induction by interleukin 4 and inhibition by interferon .gamma., Proc. Natl. Acad. Sci. (U.S.A.) 86:2829-2833 (1989).  
 Bollag et al., Homologous recombination in mammalian cells, Annu. Rev. Genet. 23:199-225 (1989).  
 Bruggemann et al., Human antibody production in transgenic mice: expression from 100 kb of the human IgH locus, Eur. J. Immunol. 21:1323-1326 (1991).  
 Bruggemann et al., A repertoire of monoclonal antibodies with human heavy chains from transgenic mice, Proc. Natl. Acad. Sci. USA 86:6709-6713 (1989).  
 Bucchini et al., Rearrangement of a chicken immunoglobulin gene occurs in the lymphoid lineage of transgenic mice, Nature 326:409-411 (1987).

Capecchi, The new mouse genetics: Altering the genome by gene targeting, TIG 5:70-76 (1989).

Capecchi, Altering the genome by homologous recombination, Science 244:1288-1292 (1989).

Coffman et al., T cell activity that enhances polyclonal IgE production and its inhibition by interferon- $\gamma$ , J. Immunol. 136:949-954 (1986).

Coffman et al., A mouse T cell product that preferentially enhances IgA production, J. Immunol. 139:3685-3690 (1987).

Doetschman et al., Targetted correction of a mutant HPRT gene in mouse embryonic stem cells, Nature 330:576-578 (1987).

Durdik et al., Isotype switching by a microinjected  $\mu$  immunoglobulin heavy chain gene in transgenic mice, Proc. Natl. Acad. Sci. USA 86:2346-2350 (1989).

Esser and Radbruch, Rapid induction of transcription of unrearranged S $\gamma$ 1 switch regions in activated murine B cells by interleukin 4, The EMBO J. 8:483-488 (1989).

Ferrier et al., Separate elements control DJ and VDJ rearrangement in a transgenic recombination substrate, The EMBO J. 9:117-125 (1990).

Forni, extensive splenic B cell activation in IgM-transgenic mice, Eur. J. Immunol. 20:983-989 (1990).

Gerstein et al., Isotype switching of an immunoglobulin heavy chain transgene occurs by dna recombination between different chromosomes, Cell 63:537-548 (1990).

Goodhardt et al., Rearrangement and expression of rabbit immunoglobulin  $\kappa$  light chain gene in transgenic mice, Proc. Natl. Acad. Sci. (U.S.A.) 84:4229-4233 (1987).

Gordon, Transgenic mice in immunology, The Mount Sinai Journal of Medicine 53:223-231 (1986).

Hagman et al, Inhibition of immunoglobulin gene rearrangement by the expression of a  $\lambda$ 2 transgene, J. Exp. Med. 169:1911-1929 (1989).

Ichihara et al., Organization of human immunoglobulin heavy chain diversity gene loci, The EMBO J. 7:4141-4150 (1988).

Iglesias et al., Expression of immunoglobulin delta chain causes allelic exclusion in transgenic mice, Nature 330:482-484 (1987).

James and Bell, Human monoclonal antibody production current status and future prospects, J. of Immunol. Methods 100:5-40 (1987).

Jasin and Berg, Homologous integration in mammalian cells without target gene selection, Genes & Development 2:1353-1363 (1988).

Kenny et al., Alternation of the B cell surface phenotype, immune response to phosphocholine and the B cell repertoire in M167  $\alpha$  plus  $\kappa$  transgenic mice, J. of Immunol. 142:4466-4474 (1989).

Jung et al., Shutdown of class switching recombination by deletion of a switch region control element, Science 259:984-987 (1993).

Kitamura et al., A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin  $\mu$  chain gene, Nature 350:423-426 (1991).

Koller and Smithies, Inactivating the  $\beta$ 2-microglobulin locus in mouse embryonic stem cells by homologous recombination, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989).

Lin et al., Recombination in mouse L cells between DNA introduced into cells and homologous chromosomal sequences, Proc. Natl. Acad. Sci. USA 82:1391-1395 (1985).

Linton et al., Primary antibody-forming cells secondary B cells are generated from separate precursor cell subpopulations, Cell 59:1049-1059 (1989).

Lo et al., Expression of mouse IgA by transgenic mice, pigs and sheep, Eur. J. Immunol. 21:1001-1006 (1991).

Lorenz et al., Physical map of the human immunoglobulin k locus and its implications for the mechanisms of V $\subscript{K}$ -J $\subscript{K}$  rearrangement, Nucl. Acids Res. 15:9667-9676 (1987).

Lutzker and Alt, Structure and expression of germ line immunoglobulin  $\gamma$ 2b transcripts, Mol. Cell. Biol. 8:1849-1852 (1988).

Mansour et al., Disruption of the proto-oncogene int-2 in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes, Nature 336:348-352 (1988).

Mills et al., Sequences of human immunoglobulin switch regions: implications for recombination and transcription, Nucl. Acids. Res. 18:7305-7316 (1991).

Mills et al. DNase I hypersensitive sites in the chromatin of human  $\mu$ .

immunoglobulin heavy-chain genes, *Nature* 306:809-812 (1983).

Mowatt et al., DNA sequence of the murine .delta.1 switch segment reveals novel structural elements, *J. Immunol.* 136:2674-2683 (1986).

Muller et al., Membrane-bound IgM Obstructs B cell development in transgenic mice, *Eur. J. Immunol.* 19:923-928 (1989).

Murray & Szostack, Construction of artificial chromosomes in yeast, *Nature* 305:189-193 (1983).

Neuberger et al., Isotype exclusion and transgene down-regulation in immunoglobulin-.lambda. transgenic mice, *Nature* 338:350-352 (1989).

Nikaido et al., Nucleotide sequences of switch regions of immunoglobulin C and C genes and their comparison, *J. Biol. Chem.* 257:7322-7329 (1982).

Nikaido et al., Switch region of immunoglobulin C.mu. gene is composed of simple tandem repetitive sequences, *Nature* 292:845-848 (1981).

Nussenzweig et al., A human immunoglobulin gene reduces the incidence of lymphomas in c-Myc-bearing transgenic mice, *Nature* 336:446-450 (1988).

Nussenzweig et al., Allelic exclusion in transgenic mice carrying mutant human IgM genes *J. Exp. Med.* 167:1969 (1988).

Oettinger et al., RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination, *Science* 248:1517-1523 (1990).

Petters, transgenic mice in immunological research, *Vet. Immunol. Immunopath.* 17:267-278 (1987).

Rabbits et al., Human immunoglobulin heavy chain genes: evolutionary comparisons of C.mu., C.delta. and C.gamma. genes and associated switch sequences, *Nucl. Acids Res.* 9:4509-4524 (1981).

Rath et al., Quantitative analysis of idiotypic mimicry and allelic exclusion in mice with a .mu. Ig Transgene, *J. of Immunol.* 143:2074-2080 (1989).

Rath et al., B cell abnormalities induced by a .mu. ig transgene extend to L chain isotype usage, *J. of Immunol.* 146:2841 (1991).

Ravetch et al., Evolutionary approach to the question of immunoglobulin heavy chain switching: Evidence from cloned human and mouse genes, *Proc. Natl. Acad. Sci. (U.S.A.)* 77:6734-6738 (1980).

Reid et al., A single DNA response element can confer inducibility by both .alpha.-and .gamma.-interferons, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:840-844 (1989).

Ritchie et al., Allelic exclusion and control of endogenous immunoglobulin gene rearrangement in .kappa. transgenic mice, *Nature* 312:517-520 (1984).

Rothman et al., Structure and expression of germline immunoglobulin .gamma.3 heavy chain gene transcripts: implications for mitogen and lymphokine directed class-switching, *Intl. Immunol.* 2:621-627 (1990).

Rusconi et al., Transmission and expression of a specific pair of rearranged immunoglobulin .mu. and .kappa. genes in a transgenic mouse line, *Nature* 314:330-334 (1985).

Sato et al., Physical linkage of a variable region segment and the joining region segment of the human immunoglobulin heavy chain locus, *Biochem. Biophys. Res. Comm.* 154:264-271 (1988).

Sevidy and Sharp, Positive genetic selection for gene disruption in mammalian cells by homologous recombination, *Proc. Natl. Acad. Sci. USA* 86:227-231 (1989).

Shimizu et al., Trans-splicing as a possible molecular mechanism for the multiple isotype expression of the immunoglobulin gene, *J. Exp. Med.* 173:1385-1393 (1991).

Shimizu et al., Immunoglobulin double-isotype expression by trans-mRNA in a human immunoglobulin transgenic mouse, *Proc. Natl. Acad. Sci. USA* 86:8020-8023 (1989).

Sideras et al., Production of sterile transcripts of C.gamma. genes in an IgM-producing human neoplastic B cell line that switches to IgG-producing cells, *Intl. Immunol.* 1: 631-642 (1989).

Siebenlist et al., Human immunoglobulin D segments encoded in tandem multigenic families, *Nature* 294:631-635 (1981).

Smithies et al., Insertion of DNA sequences into the human chromosomal .beta.-globulin locus by homologous recombination, *Nature* 317:230-234 (1985).

Snapper et al., Interferon-.gamma. and B cell stimulatory factor-1 reciprocally regulate Ig isotype production, *Science* 236:944-947 (1987).

Song et al., Accurate modification of a chromosomal plasmid by homologous recombination in human cells, *Proc. Natl. Acad. Sci. USA* 84:6820-6824 (1987).

Soriano et al., Targeted disruption of the c-src protooncogene leads to osteopetrosis in mice, *Cell* 64:693-702 (1991).

Stavnezer et al., Immunoglobulin heavy-chain switching may be directed by prior induction of transcripts from constant-region genes, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:7704-7708 (1988).

Storb, Immunoglobulin gene analysis in transgenic mice, in *Immunoglobulin Genes*, Academic Press Limited, pp. 303-326 (1989).

Storb et al., Expression, allelic exclusion and somatic mutation of mouse immunoglobulin kappa genes, *Immunological Reviews* 89:85-102 (1986).

Szurek et al., Complete nucleotide sequence of the murine .gamma.3 switch region and analysis of switch recombinant ion in two .gamma.3-expressing hybridomas, *J. Immunol.* 135:620-626 (1985).

Tahara et al., HLA antibody responses in HLA class I transgenic mice, *Immunogenetics* 32:351-360 (1990).

Taussig et al., Regulation of immunoglobulin gene rearrangement and expresion, *immunology today* 10:143-146 (1989).

Thomas and Capecchi, Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells, *Cell* 51:503-512 (1987).

Thomas et al., High frequency targeting of genes to specific sites in the mammalian genome, *Cell* 44:419-428 (1986).

Uhlmann and Peyman, Antisense Oligonucleotides: A new therapeutic principle, *Chemical Reviews* 90:544-584 (1990).

Weaver et al., A transgenic immunoglobulin Mu gene prevents rearrangement of endogenous genes, *Cell* 42:117-127 (1985).

Yamamura et al., Cell-type-specific and regulated expression of a human .lambda.1 heavy-chain immunoglobulin gene in transgenic mice, *Proc. Natl. Acad. Sci. USA* 83:2152-2156 (1986).

Yancopoulos and Alt, Regulation of the assembly and expression of variable-region genes, *Ann. Rev. Immunol.* 4:339-368 (1986).

Yancopoulos and Alt, Developmentally controlled and tissue-specific expression of unrearranged V.sub.H gene segments, *Cell* 40:271-281 (1985).

Yasui et al., Class switch from .mu. to .delta. is mediated by homologous recombination between .sigma..sub..mu. and .epsilon..sub..mu. sequences in human immunoglobulin gene loci, *Eur. J. Immunol.* 19:1399-1403 (1989).

Zijlstra et al., Germ-line transmission of a disrupted .beta..sub.2 -microglobulin gene produced by homologous recombination in embryonic stem cells, *Nature* 342:435-438 (1989).

Zimmer and Gruss, Production of chimaeric mice containing embryonic stem (ES) cells carrying a homoeobox Hox 1.1 allele mutated by homologous recombination, *Nature* 338:150-153 (1989).

Buttin, Exogenous Ig gene rearrangement in transgenic mice: a new strategy for human monoclonal antibody production? *TIG--vol. 3, No. 8 (Aug. 1987)*.

Green et al., Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs, *Nature Genetics* 7:13-21 (1994).

Hofker et al., Complete physical map of the human immunoglobulin heavy chain constant region gene complex, *Proc. Natl. Acad. Sci. USA* 86:5567-5571 (1989).

Humphries et al., A new human immunoglobulin V.sub.H family preferentially rearranged in immature B-cell tumours, *Nature* 331:446-449 (1988).

Jaenisch, Transgenic Animals, *Science* 240:1468-1474 (1988).

Jakovovits et al., Analysis of homozygous mutant chimeric mice: Deletion of the immunoglobulin heavy-chain joining region blocks B-cell development and antibody production, *Proc. Natl. Acad. Sci. USA* 90:2551-2555 (1993).

Lonberg et al., Antigen-specific human antibodies from mice comprising four distinct genetic modifications, *Nature* 368:856-859 (1994).

Miller et al., Structural alterations in J regions of mouse immunoglobulin .lambda. genes are associated with differnetial gene expression, *Nature* 295:428-430 (1982).

Morrison, Success in specification, *Nature* 368:812-813 (1994).

Pettersson et al., A second B cell-specific enhancer 3' of the immunoglobulin heavy-chain locus, *Nature* 344:165-168 (1990).

Scangos and Bieberich, Gene transfer into mice, *Advances in Genetics* 24: 285-322 (1987).

Stites et al., *Basic & Clinical Immunology*, p. 50 (1984).

Tanaka et al., An antisense oligonucleotide complementary to a sequence in I.gamma.2b Increase .gamma.2b germline transcripts, stimulates B cell DNA

synthesis, and inhibits immunoglobulin secretion, The Journal of Experimental Medicine 175:597-607 (1992).

Taki et al., Targeted insertion of a variable region gene into the immunoglobulin heavy chain locus, Science 262:1268-1271 (1993).

Taylor et al., Human immunoglobulin transgenes undergo rearrangement, somatic mutation and class switching in mice that lack endogenous IgM, International Immunology 6:579-591 (1994).

Vlasov et al., Arrest of immunoglobulin G mRNA translation in vitro with an alkylating antisense oligonucleotide derivative, Chemical Abstracts, p. 28, 112:229433X (1990).

Weiss, Mice making human-like antibodies, The Washington Post, Apr. 28, 1994.

ART-UNIT: 184

PRIMARY-EXAMINER: Ziska; Suzanne E.

ATTY-AGENT-FIRM: Townsend and Townsend and Crew LLP

#### ABSTRACT:

The invention relates to transgenic non-human animals capable of producing heterologous antibodies and transgenic non-human animals having inactivated endogenous immunoglobulin genes. In one aspect of the invention, endogenous immunoglobulin genes are suppressed by antisense polynucleotides and/or by antiserum directed against endogenous immunoglobulins. Heterologous antibodies are encoded by immunoglobulin genes not normally found in the genome of that species of non-human animal. In one aspect of the invention, one or more transgenes containing sequences of unrearranged heterologous human immunoglobulin heavy chains are introduced into a non-human animal thereby forming a transgenic animal capable of functionally rearranging transgenic immunoglobulin sequences and producing a repertoire of antibodies of various isotypes encoded by human immunoglobulin genes. Such heterologous human antibodies are produced in B-cells which are thereafter immortalized, e.g., by fusing with an immortalizing cell line such as a myeloma or by manipulating such B-cells by other techniques to perpetuate a cell line capable of producing a monoclonal heterologous antibody. The invention also relates to heavy and light chain immunoglobulin transgenes for making such transgenic non-human animals as well as methods and vectors for disrupting endogenous immunoglobulin loci in the transgenic animal.

21 Claims, 57 Drawing figures